# The Nature of Resistance of the 'B.9' Apple Rootstock to Fire Blight

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**Abstract**

**Rising production costs, associated with replacement of high-density plantings, along with lack of efficient control, especially where streptomycin resistant *Erwinia amylovora* strains have developed, make the identification of resistant apple rootstocks a high priority in the battle to control fire blight. B.9 rootstocks have exhibited a high level of fire blight resistance (comparable to the resistance of the Geneva® rootstocks), in several field trials. This finding contrasts with the susceptible phenotype that ungrafted B.9 rootstock liners have displayed in previous greenhouse tests. Irregularities in resistance may result from genotypic variation between nursery sources of B.9 rootstock in Europe and the United States. Using apple specific microsatellite markers, B.9 DNA from both sources was examined for variation and compared against parental cultivars. At this time, results have failed to identify any genetic variation between the two sources that would lead to a resistant phenotype. Field and greenhouse trials were conducted to determine if resistance was a product of rootstock growth conditions, or an effect of grafting. By focusing on progression of bacteria through asymptomatic tissue, we hoped to elucidate the mechanism by which grafting influences bacterial entry and colonization of the rootstock. Four rootstock clones, B.9 (US), B.9 (EUR), M.9, and G.16®, as ungrafted liners and grafted with four scion cultivars (Gala, Jonagold, Gingergold, and Red Yorking) were planted in the greenhouse and the field. Trees were inoculated, and bark sections were analyzed using PCR for presence of *E. amylovora*. Ungrafted B.9 liners from European and US sources displayed similar levels of susceptibility in greenhouse and field settings, indicating their origin does not play a role in resistance. In grafted trees, bacteria were isolated from above and below the union in all rootstocks tested. These results indicate resistance probably results from a suppressive effect on bacterial multiplication by the grafted B.9 rootstock.**

## INTRODUCTION

Fire blight, caused by the Enterobacterium *Erwinia amylovora*, has three main modes of infection in apple (*Malus* x *domestica*); blossom blight, shoot blight, and rootstock blight. Rootstock blight, though sporadic in occurrence, is the most lethal manifestation of fire blight and can be particularly damaging in terms of replacement costs and loss of productivity. The fire blight bacterium may infect the rootstock in several ways, but the most difficult to control and predict is the progression of bacteria, from the original point of infection in the scion, through asymptomatic tissue directly into the rootstock (Momol et al., 1998; Norelli et al., 2003). Losses due to rootstock blight dramatically increase when susceptible scion cultivars, such as 'Gala', are grafted on susceptible rootstocks such as 'M.9' or 'M.26'. For this reason identification of rootstocks with dependable, confirmed resistance to fire blight is of great importance. One potentially resistant rootstock is 'Budagovsky 9' ('B.9') a cold hardy cultivar horticulturally similar to 'M.9'. Concerns exist, however, about the resistant nature of this rootstock. In greenhouse tests ungrafted 'B.9' rootstock liners challenged with *E. amylovora* display high susceptibility to fire blight (Norelli et al., 2003). Conversely, in multiple field trials performed between 2000 and 2002, trees grafted on B.9 displayed high levels of field resistance to rootstock blight. During one such trial, in 2002, 'B.9'

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suffered 0% tree mortality compared with 86% and 38% tree mortality for 'M.9' and 'M.26' respectively (Aldwinckle unpublished data, 2003).

Inconsistencies in resistance evaluations could result from genetic differences at the source of propagation material. For the US market, 'B.9' is available from two source locations, Treco Nursery in Oregon, and Janssen Bros. Nursery in The Netherlands. Several US growers have observed morphological variation in plant material from these two sources (Ken Davis and Neal Manly, pers. commun., 2003). Genotypic differences in plant material may therefore be responsible for the resistant phenotype observed in field plantings. Another explanation for resistance involves interactions at the graft union preventing bacterial progression into the rootstock. Rootstock effects on scion susceptibility have long been in question; there have been anecdotal reports that 'M.7' increases fire blight resistance in certain cultivars. Recently Jensen et al. (2003), have linked this increase in resistance to differential gene expression patterns in cultivars grafted on 'M.7'. As with 'M.7' it is possible that 'B.9' exerts an effect on the scion cultivar preventing bacterial progression and/or colonization of the rootstock. Experiments were conducted to track bacterial movement through the scion cultivar into the rootstock to determine whether 'B.9' exerts an effect on bacterial movement into the rootstock.

'B.9' is comparable in some aspects to 'M.9', and although it may not be as productive as 'M.9', it would nevertheless be a promising substitute when highly susceptible scion cultivars are planted. Before further recommendations for 'B.9' can be made confirmation of its resistance phenotype and genetic uniformity must be determined.

## MATERIALS AND METHODS

DNA fingerprinting analysis was performed to determine if 'B.9' planting material propagated from the two sources was genetically identical. DNA was extracted from 'B.9', 'M.8' (a known parent of 'B.9'), as well as four distinct Russian red leaved crab apple cultivars maintained at the USDA national apple clonal repository, to represent the unidentified parent of 'B.9' in our analysis. 'M.9' and 'Robusta 5' were analyzed simultaneously as controls. Microsatellite markers (single sequence repeats, SSR's) were obtained from the following sources: Swiss Federal Institute of Technology (CH), HortResearch New Zealand (NZ), and the Plant Genetic Resources Unit USDA-ARS (GD) (Liebhard et al., 2002; Guilford et al., 1997; Hokanson et al., 1998). In total, twenty-four microsatellite markers, distributed over apple's seventeen chromosomes, were evaluated using the Genescan310 DNA sequencer. Data were further analyzed using the NTSYSpc2.1 software program identifying phylogenic relationships.

Bacterial migration was investigated using four rootstock clones, 'B.9' (US), 'B.9' (EUR), 'M.9' and 'G.16'®, as ungrafted liners and grafted with four scion cultivars ('Gala', 'Jonagold', 'Gingergold' and 'Red Yorking'). Trees were planted in a greenhouse and in an orchard setting in 2002. In 2003, grafted trees were inoculated 100 cm above the graft union with a $5x10^8$ cfu/ml suspension of *E. amylovora* strain 4001A ($Rf^r/Nal^r$), bisecting the two youngest leaves with scissors dipped in inoculum (Momol et al., 1998). After 6 weeks to allow time for bacterial migration and establishment in the rootstock, bark samples were collected using a cork borer. Samples were taken 50 cm above the graft union and 5 cm above and below the graft union to monitor bacterial migration. Bark samples were ground in 2 ml phosphate buffer, and 100 µl of grindate was plated on Luria-Bertini medium amended with rifampicin and naladixic acid. Plates were incubated at 28°C for 48 h and subsequently washed with 1 ml sterile distilled water. Presence of *E. amylovora* was confirmed using polymerase chain reaction (PCR). PCR was carried out using 80 µl reactions with approximately 5 ml of template DNA, and primers A and B, which amplify a 1KB *Pst*I gene fragment from pEa29 (Bereswill et al., 1992).

In order to verify that resistance was not an environmental effect, since susceptibility was observed in ungrafted rootstocks in the greenhouse, and resistance was

observed in grafted rootstocks in orchard plantings, ungrafted rootstocks were screened simultaneously in the field and the greenhouse for susceptibility to fire blight. Ungrafted rootstock liners, 'B.9' (US), 'B.9' (EUR), 'M.9' and 'G.16'® were inoculated and % lesion length, determined as a percent of current year's growth, was recorded after the lesion ceased extension.

## RESULTS AND DISCUSSION

Microsatellite data, obtained from twenty-four distinct polymorphic loci, suggest that 'B.9' from the two source locations was not genetically dissimilar, supporting a clonal relationship between planting material from these two sources (Fig. 1). These data present persuasive evidence that source material was not a determining factor in resistance. Although not genetically dissimilar it is possible that observed phenotypic differences are attributable to 'B.9' EUR/US belonging to different "clones" of the same cultivar, similar to phenotypically different 'M.9' clones. Recent work by Monte-Corvo et al. (2001) has shown that clones of the pear cultivar 'Rocha' displayed no detectable polymorphisms, when tested with five molecular marker systems including microsatellites, despite obvious phenotypic differences. This finding supported previous work by Gianfranceschi et al. (1998), which failed to differentiate between two clones of the apple cultivar 'Red Delicious' using 16 microsatellite markers. Data however attribute both 'B.9' sources with similar levels of fire blight susceptibility/resistance, and therefore it is concluded that clone differentiation is not responsible for the observed resistant phenotype.

Ungrafted rootstock liners were inoculated with *E. amylovora* in the field and in the greenhouse, and lesion and shoot lengths were recorded (Fig. 2). 'B.9' (EUR) and 'B.9' (US) sustained similar levels of infection in the field (53% and 41% in the field, respectively) and the greenhouse, (33% for both sources). This evidence supports microsatellite observations that 'B.9' from European and US sources are clonal and that any differences between plant material from these sources, i.e. somatic mutation, has limited effect on susceptibility to fire blight in grafted and ungrafted rootstocks. Ungrafted 'B.9' liners were also shown in both field and greenhouse screens to be susceptible to fire blight with susceptibility intermediate between 'G.16'® (resistant) and 'M.9' (highly susceptible). This indicated that resistance is associated with being grafted to a scion and is not a product of environmental conditions or screening location.

In grafted trees bacteria were isolated from all the rootstocks tested, 'B.9' (US), 'B.9' (EUR), 'M.9' and 'G.16'®, indicating the graft union did not have an exclusionary effect with these rootstocks. This finding contrasts with the hypothesis that the graft union may act as a barrier to bacterial movement. It appears that bacteria are migrating from the point of inoculation into 'B.9' rootstocks. It is important to note that although bacteria are migrating into rootstock tissue they do not cause disease in all rootstocks. Our current hypothesis is that grafted 'B.9' rootstocks have a suppressive effect on bacterial growth, and that gene expression in grafted and ungrafted rootstock tissue may differ.

## CONCLUSIONS AND PERSPECTIVES

The basis for resistance in grafted B.9 rootstocks is not clearly understood. Protein analysis of 'B.9' when utilized as a rootstock or a scion cultivar may provide insight into differentially expressed proteins responsible for its resistant phenotype as a rootstock. If resistance can be confirmed and its cause determined, it could not only support recommendation of 'B.9' as a resistant rootstock but also aid in fire blight resistant rootstock breeding and genetic engineering. The use of marker selection derived from protein expression could greatly accelerate resistance breeding.

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**Literature Cited**

Bereswill, S., Pahl, A., Belleman, P., Zellwe, W. and Geider, K. 1992. Sensitive and species-specific detection of *Erwinia amylovora* by polymerase chain reaction analysis. Appl. Environ. Microbiol. 59:3522-3526.

Gianfranceschi, L., Seglias, N., Tarchini, R., Komjanc, M. and Gessler, C. 1998. Simple sequence repeats for the genetic analysis of apple. Theor. Appl. Genet. 96:1069-1076.

Guilford, P., Prakash, S., Zhu, J.M., Rikkerink, E., Gardiner, S., Bassett, H. and Forster, R. 1997. Microsatellites in *Malus* x *domestica* (apple): abundance, polymorphism and cultivar identification. Theor. Appl. Genet. 94:249-254.

Hokanson, S.C., Szewc-McFaden, A.K., Lamboy, W.F. and McFerson, J.R. 1998. Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationship in a *Malus* x *domestica* Borkh. core subset collection. Theor. Appl. Genet. 97:671-683.

Jensen, P.J., Rytter, J., Detwiler, E.A., Travis, J.W. and McNellis, T.W. 2003. Rootstock effects on gene expression patterns in apple tree scions. Plant Mol. Biol. 53:493-511.

Liebhard, R., Gianfranceschi, L., Koller, B., Ryder, C.D., Tarchini, R., van de Weg, E. and Gessler, C. 2002. Development and characterization of 140 new microsatellites in apple (*Malus* x *domestica* Borkh.). Mol. Breed. 10:217-241.

Momol, M.T., Norelli, J.L., Piccioni, D.E., Momol, E.A., Gustafson, H.L., Cummins, J.N. and Aldwinckle, H.S. 1998. Internal movement of *Erwinia amylovora* through symptomless apple scion tissues into the rootstock. Plant Dis. 82:646-650.

Monte-Corvo, L., Goulao, L. and Oliveira, C. 2001. ISSR analysis of cultivars of pear and suitability of molecular markers for clone discrimination. J. Amer. Soc. Hort. Sci. 126:517-522.

Norelli, J.L., Holleran, H.T., Johnson, W.C., Robinson, T.L. and Aldwinckle, H.S. 2003. Resistance of Geneva and other apple rootstocks to *Erwinia amylovora*. Plant Dis. 87:26-32.

Norelli, J.L., Jones, A.L. and Aldwinckle, H.S. 2003. Fire blight management in the 21st century: using new technologies that enhance host resistance in apple. Plant Dis. 88:756-765.

## Figures

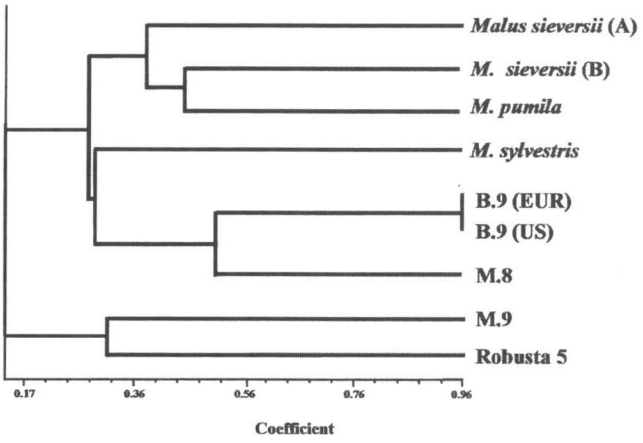


Fig. 1. Microsatellite analysis of B.9 EUR/US rootstock and related genotypes.

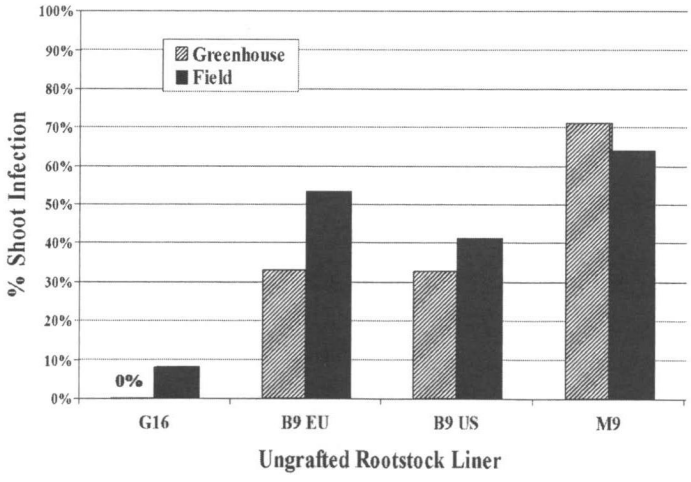


Fig. 2. Percent shoot infection of ungrafted rootstock liners inoculated with *E. amylovora* strain 4001A.